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Search Results - Record(s) 1 through 10 of 14 returned.

☑ 1. 6379929 . 19 Nov 97; 30 Apr 02. Chip-based isothermal amplification devices and methods. Burns; Mark A., et al. 435/91.2; 435/91.1 436/501. C12P019/34.

☐ 2. 6271022 . 26 Mar 99; 07 Aug 01. Device for incubating and monitoring multiwell assays. Bochner; Barry. 435/287.3; 356/388 356/395 382/128 382/133 422/65 422/82.05 435/288.7. C12M001/34.

☐ 3. 6269313 . 02 Mar 00; 31 Jul 01. Method for predicting the presence of congenital and therapeutic conditions from coagulation screening assays. Givens; Thomas B., et al. 702/22; 702/27 702/28 702/30 702/32 703/11 703/12. G01N033/49.

☐ 4. 6183693 . 27 Feb 98; 06 Feb 01. Random access slide stainer with independent slide heating regulation. Bogen; Steven A., et al. 422/64; 422/67. G01N001/31.

☑ 5. 6132996 . 08 Feb 99; 17 Oct 00. Thermocycling apparatus and method. Hunicke-Smith; Scott P.. 435/91.2; 435/285.1 435/287.2 435/287.3 435/91.5. C12P019/34.

☐ 6. 6130098 . 26 Sep 97; 10 Oct 00. Moving microdroplets. Handique; Kalyan, et al. 436/180; 366/DIG.2 422/100 422/102 422/56 422/58 422/81 422/82 436/52 436/53. G01N001/10 G01N001/18.

☐ 7. 6054263 . 14 Jul 98; 25 Apr 00. Thermal cycler including a temperature gradient block. Danssaert; John Lewis, et al. 435/4; 165/11.1 165/201 165/253 165/267 422/109 422/50 422/99 435/286.1 435/303.1 435/6 435/809 436/174. C12M003/00.

☐ 8. 5985651 . 16 Jun 97; 16 Nov 99. Thermocycling apparatus and method. Hunicke-Smith; Scott P.. 435/285.1; 165/177 165/183 219/201 219/535 422/81 435/286.5 435/287.2. C12M003/00.

☐ 9. 5866004 . 08 Sep 95; 02 Feb 99. Automated supercritical fluid extraction method and apparatus. Houck; Raymond K., et al. 210/634; 210/136 210/143 210/180 210/181 210/511 422/102 422/63 422/64 422/69 436/178. B01D011/00.

☐ 10. 5779981 . 19 Apr 96; 14 Jul 98. Thermal cycler including a temperature gradient block. Danssaert; John Lewis, et al. 422/99; 422/104 422/63 422/67 435/285.1 435/286.1 435/286.2 435/288.4 435/303.1 435/809. B01L007/00.

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Search Results - Record(s) 11 through 14 of 14 returned.

☑ 11. 5525300 . 20 Oct 93; 11 Jun 96. Thermal cycler including a temperature gradient block. Danssaert; John L., et al. 422/99; 422/104 422/63 422/67 435/285.1 435/286.1 435/286.2 435/288.4 435/303.1 435/809. B01L007/00.

☒ 12. 5176203 . 31 Jul 90; 05 Jan 93. Apparatus for repeated automatic execution of a thermal cycle for treatment of samples. Larzul; M. Daniel. 165/61; 417/50 422/189 422/198 435/286.1. F25B029/00 C12M001/38.

☐ 13. 4058367 . 19 Aug 76; 15 Nov 77. Automatic asynchronous fluid processing apparatus. Gilford; Saul R.. 422/63; 422/67. G01N001/14 G01N033/16.

☐ 14. 3739629 . 09 Feb 71; 19 Jun 73. PSYCHROMETER AND METHOD. Campbell; Eric C.. 73/77;. G01n025/62.

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Term	Documents
CYCL$3	0
CYCL.DWPI,EPAB,JPAB,USPT.	1627
CYCLA.DWPI,EPAB,JPAB,USPT.	15
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CYCLAC.DWPI,EPAB,JPAB,USPT.	2
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CYCLAGE.DWPI,EPAB,JPAB,USPT.	1
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L3: Entry 5 of 14 File: USPT Oct 17, 2000

DOCUMENT-IDENTIFIER: US 6132996 A
TITLE: Thermocycling apparatus and method

Abstract Text (1):
A thermocycling apparatus comprising a plurality of capillaries for moving DNA-containing samples between two or more discrete zones maintained at selected elevated temperatures.

Brief Summary Text (9):
Most currently-available thermocyclers are so-called "block thermocyclers". Such block thermocyclers have contributed much to the success of polymerase chain reaction (PCR; Mullis, Mullis, et al.) by allowing users a simple, convenient, and repeatable method for temperature cycling reactions. They allow very large volume reactions for preparative work, though this often requires re-optimization of the cycling parameters since temperature control is usually done on the block temperature and not the sample temperature. Due to the large sample size, block thermocyclers are relatively slow, with even the best machines (e.g., Perkin-Elmer 9600) capable of a maximal throughput of less than 1500 reactions per day. Further, block thermocyclers are typically among the most expensive in terms of per-sample reagent costs is (the Perkin-Elmer 9600 costs about $4.00 per sample).

Brief Summary Text (10):
Corbett, et al., describe a continuous serial-flow thermal cycler having a single long capillary tube wound around heating elements maintained at different temperatures. Different samples are serially passed through the same tube. The invention suffers from the disadvantages of potential sample contamination by residue left from a previous bolus, and inability to independently vary the dwell time or temperature for different samples.

Brief Summary Text (11):
Accordingly, a need exists for a rapid, efficient thermocycler. The present invention provides such a thermocycler--an apparatus capable of rapid amplification of small volume DNA samples, with minimal potential for cross-over contamination and capacity for independent regulation of the cycling parameters for each sample, at a per-sample reagent cost of less than $0.20.

Brief Summary Text (13):
The present invention includes, in one aspect, an apparatus for thermally cycling a DNA sample. The apparatus includes (i) a support and (ii) a heating unit assembly mounted on the support. The heating assembly has first and second heating elements defining first and second heating chambers, respectively, which are adapted to receive and contact a capillary tube. The capillary tube, which during operation of the apparatus contains the sample, extends through the two heating chambers. The apparatus further includes (iii) a means for maintaining the temperatures of the first and second chambers of the heating unit assembly at selected first and second elevated (i.e., above ambient) temperatures, and (iv) a means for moving the sample in the tube successively between the two chambers. The temperatures are typically selected such that one elevated temperature is effective to denature the DNA sample and the other elevated temperature is one at which DNA annealing and primer-directed DNA polymerization can occur.

Brief Summary Text (19):
A general embodiment useful for thermally cycling a plurality of DNA samples comprises a plurality of heating unit assemblies (e.g., 8 or 12) mounted on a single support (e.g., at 9 mm interval for use with a 96-well plate). The sample moving means in such an apparatus may be designed to move the samples in all capillaries in a concerted

manner, or to move them independently of one another. Further, the temperatures of the heating chambers of each heating unit assembly may be maintained independently of temperatures of the heating chambers of other heating unit assemblies. The heating unit assemblies, sample moving means and temperature maintenance means in such an apparatus may all be mounted on a single support, such as a module board. Such a module board may be used, for example, in an instrument capable of receiving a plurality of such module boards, to provide a planar array of DNA thermocyclers with a spacing corresponding

Brief Summary Text (22):
In another aspect, the present invention includes a thermocycling apparatus for thermally cycling DNA samples containing primer and DNA polymerase reagents required for primer-directed amplification of the DNA. The apparatus includes (i) a capillary assembly, which includes (a) first and second heating elements, defining first and second heating chambers, respectively, (b) a capillary tube extending through the two heating chambers, and (c) a means for introducing one such DNA sample into the capillary tube; (ii) means for maintaining the temperatures of the first and second chambers of the capillary assembly at selected first and second elevated temperatures, wherein one elevated temperature is effective to denature the DNA sample and the other elevated temperature is one at which DNA annealing and primer-directed DNA polymerization can occur; and (iii) means for moving the samples in the tubes successively between the two chambers of each capillary assembly. In a preferred embodiment, the apparatus includes plurality of such capillary assemblies.

Brief Summary Text (32):
Also included in the invention is a related apparatus for thermally cycling a DNA sample. The apparatus includes (i) a support; (ii) a heating unit assembly mounted on the support, the assembly having first and second heating elements defining first and second heating chambers, respectively, the chambers being adapted to receive and contact a capillary tube, containing the sample, extending through the two heating chambers; (iii) a temperature control system having a temperature controller electrically connected to a power delivery system and a temperature feedback system, the power delivery system being connected via leads to the heating elements, and the feedback system including temperature sensors in the heating elements, wherein the controller regulates power delivered to each heating element via the power delivery system based on signals from the feedback system, in order to maintain the temperatures of the first and second chambers of the heating unit assembly at selected first and second elevated temperatures; and (iv) a sample position control unit adapted to connect and form a seal with a first end of the capillary tube, and being effective to alter pressure at the first end, the altering of pressure being effective to move a DNA sample in the tube successively between the two chambers.

Brief Summary Text (33):
The invention further includes a method of amplifying target DNA using an apparatus such as described above. The method includes the steps of loading a sample containing target DNA, dNTPs, thermostable DNA polymerase (e.g., Tag polymerase) and primers into an apparatus as described above, and cycling the sample between at least two elevated temperatures effective to result in primer-specific amplification of the target.

Detailed Description Text (2):
I. Thermal Cycling Apparatus

Detailed Description Text (17):
B. Sample Position Control Units In embodiments where the (fluid) sample is moved between different temperature regions inside a stationary capillary, the force which moves the sample is typically a change in the pressure of the fluid (typically air) column above the sample. Such a pressure change may be effected by a number of different mechanisms, including a syringe/plunger system in combination with a linear stepper motor or DC motor, a gated pressure/vacuum supply, a cascaded solenoid system, electrostatic or piezoelectric diaphragm displacement, and "bimetallic" junction beam displacement (bending moment). Such mechanisms, which are effective to move the sample between different temperature regions, together with any electronics that may control or regulate their operation, are examples of sample position control units, or moving means.

Detailed Description Text (34):
FIG. 5 shows a preferred embodiment of the present invention. It is analogous in all respects to the apparatus described with reference to FIGS. 1A, 1B, 2, 3A, 3B, 4A and 4B, except that each capillary assembly contains 3 separate heating chambers, formed by 3 separate heating elements, 110, 112 and 114, and preferably containing 3 separate temperature sensors--one for each heating element. The third chamber of each capillary assembly provides a third temperature zone through which the DNA sample may cycled, and enables, e.g., 3-temperature polymerase chain reaction (PCR; Mullis, Mullis, et al.) employing a denaturing temperature, an annealing temperature and an extension temperature. Other relevant portions of the apparatus, such as the structures and controls that are part of the sample moving means, are of course adapted so that the sample may be moved between the three zones or regions.

Detailed Description Text (41):
II. Operation of Thermal Cycling Apparatus

Detailed Description Text (48):
Cycle parameters were downloaded through a standard RS-232 protocol which the microprocessor comes equipped to handle. In the example described, it was necessary to shift the standard RS-232 voltage levels to CMOS compatible voltages; the Harris Semiconductor HIN232 accomplished this while operating on a single 5V supply.

Detailed Description Text (54):
All events were timed using the internal Real Time Interrupt (RTI) system which was configured to increment a 16-bit counter once every 27.3 mS. This set the minimum event length as well as the maximum latency to check each channel's state and suffer no timing error. The software has 4 sequential functional modes; data entry, sample loading, sample cycling, and sample unloading.

Detailed Description Text (55):
Data entry permits the user to download new cycle parameters or use existing ones and then begin a run. The data to be downloaded typically includes times at each of the three positions for each sample (in clock ticks, 27.3 mS per tick) and number of cycles in each tube as well as "tuning" parameters. The tuning parameters are fractional amounts of the movement time which must be added during motion to ensure that the sample moves from the optical sensor, where sample detection is made, into the heated regions a few millimeters away. Note that since the times are downloaded as 16-bit unsigned integers, the longest hold time available is 23.7 mS*2.sup.16 =25.8 hours.

Detailed Description Text (56):
Lastly, the length of time required to unload the samples is downloaded. These parameters are echoed back to the user. They may then be stored in EEPROM for this run and subsequent runs. Alternatively, the user may chose parameters already in EEPROM or they may chose to enter the data again. Some parameters can be programmed to have special meaning; for example, 0 cycles specified for one channel leaves that channel completely unmoved for the entire run.

Detailed Description Text (58):
To load samples, inward motion was started and the lowest optical sensor was monitored. When fluid reached this sensor in a particular tube, motion stopped for that channel. Once all eight sensors had been tripped, motion was started for a short, fixed length of time which was downloaded with the cycle parameters. This pulled the sample from just below the optical sensor into the region of the first heater.

Detailed Description Text (60):
1. Is this channel finished cycling? If so, begin unloading process.

Detailed Description Text (65):
During this polled mode of operation, the user may modify the contents of any RAM location, query the current status of the apparatus, or reset the microcontroller. The apparatus can be queried for current run conditions or for the current program. Data returned for current run conditions includes number of cycles done, current time count, and current sample position.

Detailed Description Text (66):
For current program, the apparatus returns the number of cycles programmed, time at each heater position, and fraction of motion required to reach the next heater after tripping an optical sensor. Motion between the optical sensors is monitored to check for sensor failures. After each motion, the time taken for that motion is stored in RAM. During the next cycle, when that motion is again repeated the optical sensor is only monitored after some fraction (1-1/T2) of the previous move time has elapsed. This prevents premature stops due to faulty sensing. Similarly, if the move has taken (1+1/T1) longer than the previous move, motion is immediately stopped and the movement time is set as that for the previous correct move.

Detailed Description Text (70):
The heater drive was pulse width modulated (PWM). In this mode of operation, the duty cycle of a constant frequency (e.g., 17 kHz) square wave was modulated to control the power to the heating elements. To provide a selected amount of power to a heater or heating element, the microcontroller computed a control value, ranging from 0 to 100% of the maximum power output. The microprocessor then selected the appropriate PWM circuit (Harris Semiconductor CDP68HC68W1) and transmitted the control value to that circuit via a high speed serial communication link. The PWM circuit then modulated its output square wave to match the transmitted duty cycle. This square wave output (5 V TTL level) controlled one of eight darlington power transistor (part ULN2803A), each of which is capable of switching over 1 W of power. When the transistors were switched on (by a high level from the PWM circuit), they connected one side of the resistive heater with ground, generating heat where required.

Detailed Description Text (72):
It will be noted that in the PWM mechanism, the output voltage, and not output power, is proportional to duty cycle. This is relevant with respect to control schemes discussed in the software section below.

Detailed Description Text (76):
There eight voltage divider outputs of each row of sensors were connected to one Harris Semiconductor DG408 analog multiplexer. The inputs to these two (in the case of two-temperature cyclers) or three (in the case of three-temperature cyclers) multiplexers are gated to the output under the control of the microcontroller. The multiplexer outputs were digitized by Harris Semiconductor HI5812 12 bit analog to digital (A/D) converters. The distance between multiplexers and A/D converters did increase the amount of noise on the sensor lines. However, since the time constant of the heater/sensor assembly was on the order of a few seconds, and the microprocessor read temperatures and updated heater outputs twice per second, this random noise did not significantly affect temperature control.

Detailed Description Text (85):
An exemplary application of an apparatus constructed according to the present invention is thermal cycling of target DNA-containing samples having primer and DNA polymerase reagents required for primer-directed amplification of the DNA. Reagents typically employed in such amplification reactions include reaction buffer containing MgCl.sub.2, dNTPs, a primer set and a thermally-stable DNA polymerase (e.g., Taq polymerase). Reaction conditions for various amplifications are well known in the art (see, e.g., Mullis, Mullis, et al., Ausubel, et al.).

Detailed Description Text (86):
Samples containing the selected reagents, such as DNA target, primers, dNTPs and polymerase, are introduced into the capillaries by placing the open ends of the capillaries into vessels containing the mixture to be cycled, and applying negative pressure at the back (upper) ends of the capillaries. In an exemplary embodiment, the negative pressure is applied by withdrawal of a plunger in a syringe body, as illustrated in FIG. 3A. The plunger may be withdrawn by rotation of the threaded rod 68 in a direction effective to move the nut 70 toward the motor 58.

Detailed Description Text (87):
The volume of the samples depends on the design of the device--devices employing relatively large-diameter capillaries and/or devices employing heating elements that extend over a substantial portion of the sample-containing capillary can accommodate a larger sample than devices employing small-diameter capillaries and/or devices

employing "narrow" heating elements that extend over a relatively small portion of the sample-containing capillary. Devices designed for small-diameter capillaries have several advantages. For example, sample evaporation is minimized, cycle speed is increased and the size, power consumption and cost of components is reduced, since the total heat capacity of the "plug" of sample is smaller. The embodiments described herein are designed for sample volumes in the range of between about 0.5 and 10 .mu.l per capillary, preferably in the range of between about 0.5 and 5 .mu.l per capillary.

Detailed Description Text (88):
After loading of samples, the apparatus can begin the thermal cycling process using, for example, a protocol programmed as described in the above software sections. In a typical PCR reaction, the sample is denatured at a "first" relatively high temperature (e.g., 96.degree. C.) for several minutes, and then cycled between either two or three temperatures for a selected (e.g., 30) cycles. In a two temperature protocol, the second temperature is one at which the primers can selectively and specifically anneal to the target DNA and primer-directed DNA polymerization can occur (e.g., 60.degree. C.). In a three temperature protocol, the second temperature is one at which the primers can specifically and selectively anneal to the template DNA (e.g., 55.degree. C.) and the third temperature is one at which primer-directed polymerization can occur efficiently (e.g., 72.degree. C.).

Detailed Description Text (89):
An apparatus such as illustrated in FIG. 5, containing eight capillary assemblies, was used to PCR amplify target M13 mp template containing a yeast DNA insert using M13(-21) and M13(-48) primers and Taq polymerase for 32 cycles at 44 sec per cycle. Amplification products resolved on an agarose gel and visualized using ethidium bromide are shown in FIG. 8. Lane markers are in the first (left) lane. The next eight lanes contain the reaction products from the eight sample capillaries of the apparatus.

Detailed Description Text (90):
Other applications of the thermocycling apparatus of the present invention include cycle sequencing and restriction digests. Cycle sequencing is a variation on PCR, where the reaction is typically carried out using labeled primers.

Detailed Description Text (91):
Thermocycling devices or apparatuses of the present invention provide a number of advantages over existing thermal cyclers. Among these are the following: they have a very rapid cycle time (as short as 15-30 sec per cycle); they use small sample volumes (typically 0.5-5 .mu.l, preferably 0.5-3 .mu.l); each sample can be individually programmed for different cycle temperatures and durations; and low power consumption.

CLAIMS:

1. A method of amplifying target nucleic acid, comprising the steps of:

introducing a sample containing target nucleic acid, nucleotides, enzyme and primers into a capillary tube mounted on a support; said capillary tube having a sample position control unit, including a syringe body and a plunger movable within the syringe body, disposed in communication therewith;

maintaining first and second regions of said tube at respective first and second temperatures;

altering the pressure within said tube by moving said plunger in a reciprocating motion within said syringe body, thereby moving the sample within said tube successively between said first and second regions in a reciprocating motion, such that the sample is cycled between at least two temperatures in a manner effective to result in primer-specific amplification of the target.

3. A method of amplifying target nucleic acids, comprising the steps of:

introducing a plurality of samples, each containing target nucleic acid, nucleotides, enzyme and primers, into respective capillary tubes mounted on a support;

maintaining first and second regions of each tube at respective first and second temperatures;

moving the samples within all of said tubes in a concerted manner between said first and second regions, thereby cycling the samples between at least two temperatures in a manner effective to result in primer-specific amplification of each target.

5. A method of amplifying target nucleic acid, comprising the steps of:

introducing a sample containing target nucleic acid, nucleotides, enzyme and primers into a capillary tube mounted on a support;

operating a temperature control unit disposed in communication with a heating unit assembly, both mounted on said support, to maintain first and second regions of said tube at respective first and second temperatures;

operating a sample position control unit, mounted on said support, to move the sample within said tube between said first and second regions, thereby cycling the sample between at least two temperatures in a manner effective to result in primer-specific amplification of the target.

☐ Generate Collection

L3: Entry 11 of 14 File: USPT Jun 11, 1996

DOCUMENT-IDENTIFIER: US 5525300 A
TITLE: Thermal cycler including a temperature gradient block

Abstract Text (1):
A method in which a temperature gradient is generated across a "gradient" block, and an apparatus comprising a block across which a temperature gradient can be generated. By setting up such a gradient, multiple reaction mixtures held in wells on the gradient block can be simultaneously run at temperatures which differ only slightly, thereby permitting an optimum temperature for the reaction to be quickly identified. In a preferred embodiment the gradient block is integrated into a thermal cycler used for nucleic acid amplification reactions.

Brief Summary Text (2):
The present invention relates to a temperature cycling apparatus useful for performing nucleic acid amplification, DNA sequencing and the like which apparatus can include single or multiple heating and/or cooling blocks containing sample wells wherein a temperature gradient can be generated across a given block.

Brief Summary Text (4):
Systems which require multiple or cyclic chemical reactions to produce a desired product often require careful temperature control to produce optimal results. Such reactions include nucleic acid amplification reactions such as the polymerase chain reaction (PCR) and the ligase chain reaction (LCR). For this reason, apparatus have been developed which permit the accurate control of the temperature of reaction vessels in which such amplification reactions are performed.

Brief Summary Text (5):
For example, there are a number of thermal "cyclers" used for DNA amplification and sequencing in the prior art in which one or more temperature controlled elements or "blocks" hold the reaction mixture, and the temperature of a block is varied over time.

Brief Summary Text (6):
Another prior art system is represented by a temperature cycler in which multiple temperature controlled blocks are kept at different desired temperatures and a robotic arm is utilized to move reaction mixtures from block to block.

Brief Summary Text (8):
PCR is a technique involving multiple cycles that results in the geometric amplification of certain polynucleotide sequence each time a cycle is completed. The technique of PCR is well known to the person of average skill in the art of molecular biology. The technique of PCR is described in many books, including, PCR: A Practical Approach, M.J. McPherson, et al., IRL Press (1991), PCR Protocols: A Guide to Methods and Applications, by Innis, et al., Academic Press (1990), and PCR Technology: Principals and Applications for DNA Amplification, H.A. Erlich, Stockton Press (1989). PCR is also described in many U.S. patents, including U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188; 4,889,818; 5,075,216; 5,079,352; 5,104,792; 5,023,171; 5,091,310; and 5,066,584, which are hereby incorporated by reference.

Brief Summary Text (9):
The PCR technique typically involves the step of denaturing a polynucleotide, followed by the step of annealing at least a pair of primer oligonucleotides to the denatured polynucleotide, i.e., hybridizing the primer to the denatured polynucleotide template. After the annealing step, an enzyme with polymerase activity catalyzes synthesis of a new polynucleotide strand that incorporates the primer oligonucleotide and uses the original denatured polynucleotide as a synthesis template. This series of steps

(denaturation, primer annealing, and primer extension) constitutes a PCR cycle. As cycles are repeated, the amount of newly synthesized polynucleotide increases geometrically because the newly synthesized polynucleotides from an earlier cycle can serve as templates for synthesis in subsequent cycles. Primer oligonucleotides are typically selected in pairs that can anneal to opposite strands of a given double-stranded polynucleotide sequence so that the region between the two annealing sites is amplified.

Brief Summary Text (10):
The temperature of the reaction mixture must be varied during a PCR cycle, and consequently varied many times during a multicycle PCR experiment. For example, denaturation of DNA typically takes place at around 90.degree.-95.degree. C., annealing a primer to the denatured DNA is typically performed at around 40.degree.-60.degree. C., and the step of extending the annealed primers with a polymerase is typically performed at around 70.degree.-75.degree. C. Each of these steps has an optimal temperature for obtaining the desired result. Many experiments are required to determine the optimal temperature for each step.

Brief Summary Text (17):
To achieve this object, the invention is a method in which a temperature gradient is generated across a "gradient" block. The invention also includes an apparatus comprising a block across which a temperature gradient can be generated. By setting up such a gradient, multiple reaction mixtures can be simultaneously run at temperatures which differ only slightly, thereby permitting an optimum temperature for a given reaction to be quickly identified. In the most preferred embodiment of the invention the gradient block is integrated into a thermal cycler. By doing so, it is possible to run a series of desired reactions using the thermal cycler immediately upon identification of the optimum reaction temperature.

Drawing Description Text (3):
FIG. 1 is a perspective view of a thermal cycler incorporating the thermal gradient block of the invention;

Drawing Description Text (5):
FIG. 3 is a block diagram depicting the elements of a thermal cycler in which the thermal gradient apparatus and method of the invention may be used.

Detailed Description Text (2):
The present invention relates to a device and method for creating a thermal gradient across a block, such as a block in known thermal cyclers for PCR reactions, which enables one to simultaneously conduct a series of experiments at very close to the same temperatures. As used herein, the term "block" refers to a structure, usually metal, which can be temperature controlled and in which wells have been arranged to accept vessels containing reaction mixtures or "samples." The phrase "gradient block" as used herein is intended to describe such a block, except that a gradient block is a block across which a temperature gradient can be established. Examples of the specific manner in which such a temperature gradient can be established are discussed herein, though those skilled in the art will understand that once the advantage of having a gradient block is known, many other variations of the apparatus shown herein can be easily identified.

Detailed Description Text (3):
One particular area of utility for the present invention is in multiple block thermal cyclers. By incorporating the gradient block of the invention into a multiple block thermal cycler, it is possible to simultaneously conduct a series of reactions where the temperature at which the reactions are proceeding is varied across the gradient block. This permits the rapid determination of the optimal temperature for that particular reaction.

Detailed Description Text (5):
Various components of the cycler depicted in greater detail in FIGS. 2 and 3 can be seen in FIG. 1, i.e. display 15, keypad 16, additional blocks 17, 18 and 19 and robot arm 20 (shown in cutaway view).

Detailed Description Text (8):

Thus, those skilled in the art can readily understand how the thermal gradient block of the invention can be incorporated into known thermal cyclers.

Detailed Description Text (9):
Of course, the thermal gradient block of the invention need not necessarily be incorporated into a known cycler to be advantageously used. For example, a stand alone unit incorporating the thermal gradient block could be used in conjunction with known cyclers so that optimum reaction temperatures could be identified and then used in those cyclers.

Detailed Description Text (18):
The block diagram of FIG. 3 depicts a gradient block (labelled "second block") of the type shown in FIG. 2 as block 2 integrated into a thermal cycler having multiple heating and cooling blocks. The labels in FIG. 3 are self-explanatory, and the apparatus described by FIG. 2 differs from a known thermal cycler only with respect to the substitution of the gradient block for a non-gradient block. For PCR, the first, second and third blocks in FIG. 3 may be programmed to be maintained at a temperature range of between about 25.degree. to 99.degree. C., and are used for denaturing, annealing and extension respectively. The fourth block is generally maintained at between 4.degree. and 25.degree. C. and is used for sample storage after the PCR reaction has completed. The second block, made of brass, will be used for the annealing step.

Detailed Description Text (22):
USE OF THE GRADIENT THERMAL CYCLER FOR THE POLYMERASE CHAIN REACTION

Detailed Description Text (27):
The temperature cycling parameters used were as follows:

Detailed Description Text (32):
USE OF THE GRADIENT THERMAL CYCLER FOR THE LIGASE CHAIN REACTION

Detailed Description Text (33):
Ligase chain reaction (LCR) is a recently described DNA amplification technique that can be used to detect trace levels of known nucleic acid sequences. LCR involves a cyclic two step reaction which is performed in a DNA thermal cycler machine. The first step is a high temperature melting step in which double stranded DNA unwinds to become single stranded. The second step is a cooling step in which two sets of adjacent, complementary oligonucleotides anneal to the single stranded target DNA molecules and are ligated together by a DNA ligase enzyme. The products of ligation from one cycle serve as templates for the ligation reaction of the next cycle. Thus, LCR results in the exponential amplification of ligation products.

Detailed Description Text (35):
The materials used in this experiment were obtained from Stratagene, La Jolla, Calif. The optimal temperature for the second step of the LCR cycle, in which the oligonucleotides are annealed to the DNA target molecules, was determined empirically by the use of the gradient thermal cycler of the invention. Two sets of reactions were set up, one with a wild type template to which the oligonucleotides were complementary, and one with a mutant template that differed from the wild type template DNA sequence by one base transition. The DNA templates used in this experiment were plasmid constructs containing the pBluescriptII vector and the lac I gene. The wild-type template contained a normal lac I sequence, and the mutant template contained a C to T transition mutation at site 191 within the insert. The four oligonucleotide probes consisted of two pairs of two oligonucleotides each. The first set, A and B, were adjacent to each other and complementary to one strand of the target DNA. The second set, C and D, were complementary to the first set, and therefore occupied adjacent sites on the second strand of the target DNA. The oligonucleotide probe sequences (5' to 3') were as follows:

Detailed Description Text (37):
The mutant sequence differed from the wild type by a C to T transition at site 191. The LCR experiment was performed as follows: The following ingredients were combined in a sterile 500 .mu.l of 10XZ Pfu LCR buffer, 15 .mu.l of sterile dH.sub.2 O, 1 .mu.l (10 ng of each) of oligonucleotide mixture, 1 .mu.l (100 pg) of either the wild-type

or mutant plasmid templates or no template, and 1 .mu.l (4U) of Pfu DNA ligase enzyme. A 25 .mu.l overlay of sterile mineral oil was added to the tube. This procedure was repeated so that there were a total of 5 tubes each of either the wild type template reaction mixture or the mutant template reaction mixture. The tubes were placed in the gradient thermal cycler of the invention in positions 1, 3, 5, 7 and 8, so that at each isothermal column in the machine, there would be a wild type and a mutant template reaction. The machine was programmed to cycle between a high temperature of 92.degree. C. and the gradient block, which was varied in temperature between 56.degree. C. and 70.degree. C. The machine was programmed to move to the high temperature block for 4 minutes, then the gradient block for 3 minutes, then to move between the high temperature block and the gradient block 25 times, stopping for 1 minute at each block. The ligation chain reaction products were visualized by electrophoresis on a 6% polyacrylamide get buffered with TBE, followed by staining with ethidium bromide and photography under UV light.

Detailed Description Text (39):
The wild type template reaction produced the most intense positive signal in position 8, which corresponds to the coldest (56.degree. C.) section of the gradient block. The use of the gradient thermal cycler of the invention allowed the empirical determination of the best annealing temperature for this reaction in one experiment.

Detailed Description Text (40):
There are many modifications and variations of the thermal gradient block which can advantageously be incorporated into it or related structures. Further, multiple thermal gradient blocks could be employed as more than one block of a multi-block thermal cycler where samples are automatically moved between the various blocks, thereby allowing for multiple reactions to be operated at multiple temperatures.

Detailed Description Text (41):
The invention has been described in detail with respect to its use with PCR. However, in addition to being exceptionally useful for the determination of the optimal temperature for individual steps in a PCR procedure, the invention is also useful for determining the optimal temperature for numerous other chemical reactions. These other chemical reactions include any non-PCR nucleic acid amplification that employs an annealing step analogous to a PCR annealing step, such as ligase chain reaction (LCR) and DNA cycle sequencing. Other types of reactions for which the invention will be useful include DNA sequencing, cDNA synthesis using a cycling reaction, coupled amplification sequencing (CAS), rapid amplification of cDNA ends (RACE) and any other incubation reaction in which incubations must be accomplished at multiple temperatures.

Detailed Description Paragraph Table (1):
1 min 94.degree. C. 1 min 42-56.degree. C. (gradient block) 1 min 72.degree. C. .vertline. 1 min 94.degree. C. .vertline. 30 cycles 1 min 42-56.degree. C. (gradient block) .vertline. 8 min 72.degree. C. Storage 4.degree. C.

Other Reference Publication (1):
Advertisement for Stratagene ROBOCYCLER.TM.Temperature Cycler, 1 page, Jun., 1993.

Other Reference Publication (2):
Advertisement--MJ Research, Inc. Peltier-effect Thermal Cyclers--1page, Jul., 1993.

Other Reference Publication (10):
Advertisement--Inotech Quarter Bath Immersion Thermal Cycler--1page, undated.

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L3: Entry 1 of 14 File: USPT Apr 30, 2002

DOCUMENT-IDENTIFIER: US 6379929 B1
TITLE: Chip-based isothermal amplification devices and methods

Brief Summary Text (6):
Nucleic acid amplification techniques may be grouped according to the temperature requirements of the procedure. Certain nucleic acid amplification methods, such as the polymerase chain reaction (PCR.TM.--Saiki et al., 1985), ligase chain reaction (LCR--Wu et al., 1989; Barringer et al., 1990; Barony, 1991), transcription-based amplification (Kwoh et al., 1989) and restriction amplification (U.S. Pat. No. 5,102,784), require temperature cycling of the reaction between high denaturing temperatures and somewhat lower polymerization temperatures. In contrast, methods such as self-sustained sequence replication (3SR; Guatelli et al., 1990), the Q.beta. replicase system (Lizardi et al., 1988), and Strand Displacement Amplification (SDA--Walker et al., 1992a, 1992b; U.S. Pat. No. 5,455,166) are isothermal reactions that are conducted at a constant temperature, which is typically much lower than the reaction temperatures of temperature cycling amplification methods.

Brief Summary Text (23):
As used herein, the term "an isothermal amplification reaction" refers to a nucleic acid amplification reaction that is conducted at a substantially constant temperature. It will be understood that this definition by no means excludes certain, preferably small, variations in temperature but is rather used to differentiate the isothermal amplification techniques from other amplification techniques known in the art that basically rely on "cycling temperatures" in order to generate the amplified products. Thus, the present invention is distinguished from PCR, which fundamentally rests on the temperature cycling phenomenon.

Brief Summary Text (24):
It will be further understood that although the isothermal amplification reactions of the present invention will generally be conducted at a substantially constant temperature, the overall execution of the amplification, diagnostic or prognostic methods of the invention may nonetheless require certain steps to be conducted at different temperatures. For example, moving fluids or microdroplets through the different channels or chambers defined on the microfabricated substrate, and/or merging and mixing samples and reagents, may involve alterations in temperature, e.g., as may be achieved via the use of defined heating elements.

Brief Summary Text (82):
Those of ordinary skill in the art will further understand that other physical components of the chip fabrication will impact the temperatures effective to transport microdroplets. By way of example only, in studies using glass capillaries, it has been found that there is a minimum temperature difference required to move the droplet. For instance, if the advancing angle is 36.degree. and the receding angle is 29.degree. (with the front of the droplet being 25.degree. C.), then the back of the droplet would need to be heated to .about.60.degree. C. for a 1 mm long droplet in a 20 mm high channel. This is just one example situation.

Brief Summary Text (84):
However, the calculations of the present inventors indicated that about a 35.degree. C. difference between the front and back of a droplet will be sufficient to initiate droplet motion in a system with advancing angles of 36.degree. and receding angles of 29.degree. in a 20 mm high channel. Further studies of effective transport showed that the resulting temperature difference was -40.degree. C. between the front and back of the droplet, thus corroborating the initial determination of the requirements.

Drawing Description Text (6):

FIG. 5. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P.sub.atm is atmospheric pressure, P.sub.2 is the receding-edge internal pressure, P.sub.2 is the advancing-edge internal pressure, and .theta. is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving .theta. between 0.degree. and 90.degree., and a hydrophobic surface giving .theta. between 90.degree. and 180.degree.. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

Detailed Description Text (8):
Mixing of reactants in the channels and chambers of the DNA chip is of particular concern in isothermal amplification reactions, as mixing of reactants initiates the amplification reaction. This is not the case in PCR.TM., as all reactants required for amplification are present together in the reaction mix. PCR.TM. amplification of double-stranded targets does not begin until temperature cycling is started because until that time no single-stranded target is available to amplify. This is not the case in isothermal amplification reactions. Because strand separation is an enzymatic process in isothermal amplification, at least one of the enzyme reactants (usually the polymerase) is withheld until it is desired to begin the reaction. If the isothermal amplification reaction starts with a heat-denaturation step and the enzymes employed are not thermostable, all of the enzymes for amplification are typically withheld until the target-containing sample is cooled to the appropriate reaction temperature. The sample containing the enzyme or enzymes must be mixed with the remaining reagents in order for amplification to begin.

Detailed Description Text (68):
Once the appropriate chemicals are added to the DNA sample, the solution may be passed through several different temperatures. The mixed solution may be transported to a uniformly heated reaction chamber of the unit. Once in the chamber, the temperature of the solution may be increased using local heaters and temperature sensors. The temperature of the ends of the drops may be monitored and maintained at the same temperature to prevent the drop from leaving the reaction zone. If the drop does begin to move, local temperature gradients could quickly stabilize the drop. The cooling of the drop may be accomplished by simple conduction of the heat through the walls of the channel to ambient temperature.

Detailed Description Text (70):
The mixing chamber consists of an enlarged portion of the microchannel structure, with one or more microchannels connected to the chamber. The mixing chamber is suspended on a thin silicon nitride diaphragm. This construction allows for excellent thermal isolation, as needed for low power heat cycling of the mixture. Construction of membrane suspended structures has been demonstrated (Mastrangelo et al., 1991). The heating is effected with a set of concentric resistors (heaters) that are placed on the periphery of the mixing chamber. This design, along with the high thermal conductivity of the liquid sample, makes the chamber temperature quite uniform. Along with the heaters, temperature sensors (diodes) are constructed on the diaphragm to monitor the temperature of the mixture. The low mass construction of the chamber allows for rapid heating cycles. Temperature control may handle samples of variable volume and heat capacity. The chamber also contains a set of electrodes and heating elements to drive the mixture out of the chamber at the completion of the reaction.

Detailed Description Text (106):
FIG. 3A and FIG. 3B show a schematic of one embodiment of a device (10) to split a nanoliter-volume liquid sample and move it using external air, said device having a plurality of hydrophobic regions (hatched regions). Looking at FIG. 3A, liquid (shown as solid black) placed at the inlet (20) is drawn in by surface forces and stops in the channel at the liquid-abutting hydrophobic region (40), with overflow handled by an overflow channel and overflow outlet (30). In the embodiment shown in FIG. 3A, the from of the liquid moves by (but does not enter) a gas-intake pathway (50) that is in fluidic communication with the channel; the liquid-abutting hydrophobic region (40) causes the liquid to move to a definite location. Gas from a gas source (e.g., air from an external air source and/or pump) can then be injected (FIG. 3B, lower arrow) to split a microdroplet of length "L". The volume of the microdroplet split-off (60)

is predetermined and depends on the length "L" and the channel cross-section. To prevent the pressure of the gas (e.g., air) from acting towards the inlet side, the inlet (20) and overflow ports (30) may be blocked or may be loaded with excess water to increase the resistance to flow.

Detailed Description Text (116):
Contact angle hysteresis (the contact angle on the advancing edge of the droplet is larger than the contact angle on the retreating edge) requires a minimum temperature difference before movement will occur. The velocity of the droplet after motion begins may be approximated using the equation v=AEPd.sup.2 /32 .mu.L where AEP is the pressure difference, .mu. is the viscosity of the solution, and L is the length of the droplet. The present invention contemplates temperature differences of greater than 30.degree. C. to create movement. Studies using temperature sensors arrayed along the entire channel indicate that a differential of approximately 40.degree. C. across the droplet is sufficient to provide motion. In these studies, the channel cross-section was 20/.mu.m.times.500/.mu.m, and the volume of each of these droplets may be calculated from their lengths and is approximately 100 nanoliters for a 1 cm long droplet.

Detailed Description Text (144):
From equation 4, the change in surface tension can serve as the driving force for fluid motion. One embodiment of the invention is described as a micromechanical integrated DNA analysis technology, or MIDAT. In the MIDAT system a temperature difference between the ends of the drop will be used to produce a surface tension difference. For pure water, the change in surface tension with temperature is -0.15 dyn/cm-.degree. C. (Probstein, 1989) and is constant over the entire liquid range of water (Osipow, 1962). Because the DNA solutions being used are very dilute, the surface tension values are expected to be identical to water.

Detailed Description Text (178):
PCR.TM. was run on this chip. The reaction was carried out on the surface of this chip using a polypropylene ring cemented to the chip as the vessel walls. 20 gm of reaction mix was covered with oil to prevent evaporation and the solution was cycled through 94.degree. C., 55.degree. C., and 72.degree. C. using a digital controller (National Instruments LabView, programmed VI, Macintosh Quadra 950 computer). Using such a controller based in LabView allows change in the function and design of the controller without the expense of circuit construction. As the electrophoresis gel indicates, the oxide surface of the chip and the heaters did not damage the enzyme or inhibit the reaction; the chip results appear identical to the control run on a commercial thermocycler. Extensive biocompatibility tests indicate that the results of the reaction are very sensitive to controller settings and to the materials used for construction (Burns, 1994).

Detailed Description Text (192):
Once hybridized, the nucleic acid:primer complex is contacted with one or more enzymes that facilitate template-dependent nucleic acid synthesis. Multiple rounds of amplification, also referred to as "cycles," are conducted until a sufficient amount of amplification product is produced.

Detailed Description Text (204):
An additional feature of this method is that it does not require temperature cycling. Many amplification methods require temperature cycling in order to dissociate the target from the synthesized strand. In this method, a single temperature may be employed after denaturation has occurred. The temperature of the reaction should be high enough to set a level of stringency that minimizes non-specific binding but low enough to allow specific hybridization to the target strand. In addition proper temperature should support efficient enzyme activity. From about 37.degree. C. to about 42.degree. C. has been found to be a preferred temperature range.

Detailed Description Text (207):
Another method, called Repair Chain Reaction (RCR), involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. Target specific sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having 3' and 5'

sequences of non-specific DNA and a middle sequence of specific RNA is hybridized to DNA that is present in a sample. Upon hybridization, the reaction is treated with RNase H, and the products of the probe identified as distinctive products that are released after digestion. The original template is annealed to another cycling probe and the reaction is repeated.

Detailed Description Text (213):
Other nucleic acid amplification procedures include transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA) and 3SR Gingeras et al., PCT Application WO 88/10315, incorporated herein by reference. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a clinical sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has target specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target specific primer, followed by polymerization. The double-stranded DNA molecules are then multiply transcribed by an RNA polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNA's are reverse transcribed into single stranded DNA, which is then converted to double stranded DNA, and then transcribed once again with an RNA polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target specific sequences.

Detailed Description Text (214):
Davey et al., EPA No. 329 822 (incorporated herein by reference in its entirety) disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a template for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from the resulting DNA:RNA duplex by the action of ribonuclease H (RNase H, an RNase specific for RNA in duplex with either DNA or RNA). The resultant ssDNA is a template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to the template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow" fragment of E. coli DNA polymerase I), resulting in a double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA.

Detailed Description Text (215):
Miller et al., PCT Application WO 89/06700 (incorporated herein by reference in its entirety) disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic, i.e., new templates are not produced from the resultant RNA transcripts. Other amplification methods include "RACE" and "one-sided PCR" (Frohman, 1990 incorporated by reference).

Detailed Description Text (220):
Another method for amplification is the ligase chain reaction ("LCR"), disclosed in EPA No. 320 308, incorporated herein by reference in its entirety. In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Pat. No. 4,883,750 describes a method similar to LCR for binding probe pairs to a target sequence.

Detailed Description Text (250):
where .DELTA.T is the difference between the surface temperature and the center of the gel, S is the heat generation per volume in the gel, H is the thickness of the gel, and k is the thermal conductivity of the solution. Knowing the resistivity of the solution in the gel (.rho.), one can obtain an equation for .DELTA.T in terms of operating variables: ##EQU2##

Detailed Description Text (252):
where V is in volts. Note that this equation was derived for the specific test gel that was used and for a temperature difference of 0.1.degree. C.; the equation would need to be derived for other gel/polymer systems. Note also that, for their test gel, the equation correctly calculates that H.ltoreq.0.4 mm.

Detailed Description Text (300):
As an example of an "intelligent" system, the modules take advantage of the ability to hold a sample in reserve, while portion of the sample is being examined. The determination of a DNA template size and quantity prior to more extensive processing is an use of this capability. Size information, for example, can inform the temperature, number of cycles, and electrophoresis conditions of a cycle sequencing run.

Detailed Description Text (301):
A single source DNA is used to supply three sequencing reactions. Template DNA is amplified from the source independently for each sequencing reaction. The template is then divided into two samples and one is assayed for quality and size by gel electrophoresis. The remaining template is then treated to remove unincorporated primers and dNTPs prior to cycle sequencing. The information obtained by analysis of half of the sample is used to determine the reaction parameters of later steps. This figure is presented only as an example: alternative template preparation strategies are contemplated.

Detailed Description Text (308):
It is contemplated the invention will comprise hundreds of control and detector connections. In practice, the number of external connections may be limited by the chip size. By integrating the system with on chip electronics, it may be controlled using as little as 5 external leads. One embodiment of the invention is on-chip circuitry to control the operation of the MIDAT system. These circuits may be implemented on the same substrate as the fluidic parts. On-chip integrated control circuitry may result in a highly compact and efficient design capable of making real-time control decisions. The system may comprise a sample size and flow control circuit, temperature cycling and timing circuit, electrophoretic separation bias, data detection and transmission, and a sequencer/timer to control the overall operation. All the data will be transmitted in serial form between an external computer and the MIDAT chip.

Detailed Description Text (345):
PCR.TM. was performed using standard buffer and primer concentration conditions for Thermus aquaticus DNA polymerase enzyme (Mullis and Faloona, 1987, Artheim and Erlich, 1992). PCR.TM. temperature profiles were as follows: 94.degree. C. for 4 min, preincubation; 94.degree. C. for 1 min, 62.degree. C. for 1 min, 72.degree. C. for 1 min, 35 cycles; 72.degree. C. for 10 min, final extension is specific for a portion of the mouse Tfe3 locus and produces a 460-bp-amplified product (primer A, 5'-TAAGGTATGCCCCTGGCCAC-3' (SEQ ID NO:1); primer B, 5'-AAGGTCAGCACAGAGTCCTCA-3') (SEQ ID NO:2 (Roman et al., 1992). For each experimental run a complete 75-.mu.l reaction mixture was prepared using 100 ng of purified genomic mouse DNA as template and divided into three reactions of 25 .mu.l each. The first reaction was maintained at room temperature for 2 h; the second was reacted in a thin-wall polypropylene tube under mineral oil and cycled in a standard thermal cycler; and the third was placed on the surface of the described heater wafer within a small polypropylene ring (4 mm diameter, 1.5 mm height) and covered with light mineral oil. Wafer temperatures were determined by measuring changes in heater element resistance and were controlled by a National Instruments LabView controller and software operating on an Apple Macintosh 950. On completion of the reactions, the three samples were examined for efficiency of amplification by agarose gel electrophoresis and ethidium bromide staining.

Detailed Description Text (350):
Using microfabrication processes compatible with the construction of the thermocapillary pump channels, a thermal cycling plat-form, a gel electrophoresis chamber, and a DNA detector were fabricated and tested. PCR.TM. thermal cycling was performed on a silicon substrate using heaters and temperature sensors from the same processed wafer as the thermocapillary pump. In this thermal reaction chamber device, a group of four closely spaced heater elements were tested to ensure compatibility with the standard PCR.TM. biochemical reactions. The device successfully amplified a single-copy sequence from total genomic mouse DNA in small aqueous drops (10-25 .mu.l) placed on the processed silicon surface and covered with mineral oil to prevent evaporation. However, variations in PCR.TM. amplification efficiency as large as 4-fold were observed between repetitions of the study.

Detailed Description Text (380):
This expression indicates that any heating on the back end of the droplet (if the front remains at a lower temperature) will cause the liquid droplet to move. This was not the case experimentally, however. By way of studies using glass capillaries, it was found that there was a minimum temperature difference required to move the droplet. This effect is believed to be the result of contact angle hysteresis (CAH). In CAH, the advancing contact angle is greater than the receding contact angle resulting in a sort of back pressure which must be overcome to achieve droplet movement. CAH occurs when the interface is placed in motion (dynamic angles). To account for this effect, it was included in a steady-state (1D) model for flow. For instance, if the advancing angle is 36.degree. and the receding angle is 29.degree. (with the front of the droplet being 25.degree. C.), then the back of the droplet would need to be heated to .about.60.degree. C. for a 1 mm long droplet in a 20 .mu.m high channel. This is just one example situation.

Detailed Description Text (384):
The present calculations suggest that a .about.35.degree. C. difference between the front and back of a droplet should be sufficient to initiate droplet motion in a system with advancing angles of 36.degree. and receding angles of 29.degree. in a 20 .mu.m high channel. Experimental testing of actual devices however, showed that the front of the droplet heats relatively quickly thus reducing the temperature difference needed for movement between the front and the back of the droplet. This effect required the invention to use higher voltages to obtain droplet motion. Voltages typically in the range of .about.30.degree. Volts were found to be required to obtain motion. Further studies showed that the resulting temperature difference was .about.40.degree. C. between the front and back of the droplet thus corroborating the initial determination of the requirements.

Detailed Description Text (397):
A device fabricated with metal resistive heaters and oxide/nitride/oxide coating was tested for biological compatibility and temperature control by using PCR.TM. amplification of a known DNA template sample. The reaction was carried out on the planar device using twenty microliters of PCR.TM. reaction mix covered with mineral oil to prevent evaporation. The reaction mixture was cycled through a standard 35-cycle PCR.TM. temperature cycling regime using the integral temperature sensors linked to a programmable controller. Since the reaction volume was significantly larger than intended for the original heater design, a polypropylene ring was cemented to the heater surface to serve as a sample containment chamber. In all test cases, the presence of amplified reaction products indicated that the silicon dioxide surface and the heater design did not inhibit the reaction. Parallel amplification studies performed on a commercial PCR.TM. thermocycler gave similar results. A series of PCR.TM. compatibility tests indicated that the reaction on the device is very sensitive to controller settings and to the final surface material in contact with the sample.

Detailed Description Text (438):
Burggraf, Manz, de Roij, Widmer, "Synchronized cyclic capillary electrophoresis: a novel concept for high-performance separations using low voltages," Analytical Methods and Instrumentation, 1:55-59, 1993.

Other Reference Publication (12):
Wilding et al., "Thermal cycling and surface passivation of micromachined devices for

PCR" Clin. Chem., 41:1367-1368, 1995.